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STUDIES ON *CUTEREBRA EMASCULATOR* FITCH 1856 (DIPTERA: CUTEREBRIDAE) AND A DISCUSSION OF THE STATUS OF THE GENUS *CEPHENEMYIA* LTR. 1818[1]

BY GORDON F. BENNETT

Abstract

Cuterebra emasculator Fitch, a subcutaneous warble of the eastern striped chipmunk, *Tamias striatus* Richardson, was found in 141 of 465 chipmunks examined in Algonquin Park, Ontario. The various stages of the insect are described. The life history was studied and it was found that the larval development required 19 days. Adults emerged from the puparia in 219 (134–315) days and were short-lived. The larvae had little adverse effect on the chipmunk until after their escape from the host, when the sites frequently became secondarily infected. No case of emasculation was noted. *Cuterebra emasculator* was found to be specific for the chipmunk, *Tamias striatus*. The genus *Cephenemyia* Ltr. was compared with the genus *Cuterebra* Clk. The former was removed from the family Cuterebridae and placed in the Oestridae.

Introduction and Historical

The abundance of *Cuterebra emasculator* Fitch, 1856, in the chipmunks (*Tamias striatus* Richardson) of Algonquin Park has provided an opportunity to study the life history, incidence of parasitism, and certain host–parasite relationships. All stages in the life cycle will be described, since the egg and first two instars, as far as is known, have not been described previously and the third instar, puparium, and adult are inadequately known. This and other species of *Cuterebra* are compared with species of *Cephenemyia* to reassess the systematic position of the two genera.

For many years, all warble or bot type Diptera were included in the family Oestridae, erected by Linnaeus in 1736 for the genus *Oestrus*. The genera *Cuterebra*, erected by Clark in 1815, and *Cephenemyia*, erected by Latreille in 1818, were also placed in this family (Brauer, 7). Brauer (7) erected two new genera, *Dermatobia* and *Rogenhofera*, including them in the Oestridae. Here they remained until Austen (1) created the family Cuterebridae Aus., 1895, into which he placed the genera *Cuterebra* Clk., *Cephenemyia* Ltr., *Dermatobia* Br., *Rogenhofera* Br., and also a new genus, *Bogeria* Austen. Bau (3) and (4), divided *Bogeria* Aus. into *Bogeria* Aus. and *Pseudobogeria* Bau, 1929. Townsend (26) in 1934 considered that tribal status should be assigned to each

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of the genera *Cuterebra* Clk., *Cephenemyia* Ltr., *Dermatobia* Br., and *Pseudogametes* Birchoff in the family Cuterebridae. He listed 12 genera under these tribes in North and South America. The tribal and generic arrangement proposed by Townsend is not included here, as most of the more recent workers have not accepted his proposal.

Curran (9) in 1934 proposed a new classification, in which he accepted the family Cuterebridae Aus., but listed only four genera, *Pseudogametes* Birch., *Rogenhofera* Br., *Dermatobia* Br., and *Cuterebra* Clk., as occurring in North and South America. He considered *Bogeria* Aus. as a synonym of the genus *Cuterebra* Clk. Curran does not mention any of the other genera that Townsend (26) listed for North and South America, so it is presumed that he did not consider them to be valid and hence all species should be included in the genus *Cuterebra* Clk. Dalmat (11) accepted Curran's classification but James (15) followed Townsend (26). Sabrosky (23) considered that Curran's classification is correct and this classification has been used herein.

Cuterebra emasculator was described by Fitch (12) in 1856 from a specimen reared from a larva taken from the scrotal sac of a *Sciurus striatus* (*Tamias striatus* R.). Brauer (7) reported that Löw in the period 1856–1863 had described *Cuterebra scutellaris*, but Brauer could find no difference between *C. emasculator* and *C. scutellaris*, and therefore he placed *scutellaris* in synonomy. Coquillet (8) in 1898, after examining Fitch's original specimen, synonomized *C. emasculator* to *C. fontinella*, a form which is found in rabbits. In 1917, Townsend (25) validated Fitch's species, but he placed it in the genus *Bogeria* Aus. This species was placed in the genus *Pseudobogeria* by Bau (3) in 1929. Townsend (26) accepted this arrangement, but Curran (9) disregarded these genera entirely. Sabrosky (23) accepted Curran's classification and hence this species is termed *Cuterebra emasculator* Fitch, 1856, in the present paper.

Curran (9) omitted the genus *Cephenemyia* Ltr. in his classification of the North American Cuterebridae. Neither did he refer to it as a genus of the North American Oestridae, in which he included only the genera *Oestrus* L., *Cephalemya* Ltr., *Oedemagena* Ltr., and *Hypoderma* Clk. Townsend (26), however, placed the genus *Cephenemyia* in the tribe Cephenemyinii, family Cuterebridae, superfamily Oestroidea. James (15) in 1947 followed Townsend (26), implying however, that a difference existed between *Cephenemyia* and *Cuterebra*. LeClercq (18) in 1948, in a review of the European Oestridae, presented a new classification, in which he placed the genera *Hypoderma* and *Cephenemyia* in the Calliphoridae, and *Oestrus* in the Larvaevoridae, omitting mention of the Cuterebridae. This confused situation requires clarification, and later in the text the affinities of *Cephenemyia* with the Oestridae will be elaborated.

Materials and Methods

Field research was carried out at the Department of Lands and Forests Wildlife Research area in Algonquin Park, Ontario. The host animals were obtained in an area about eight by four miles, surrounding Lake Sasagewan.

Living chipmunks were captured, anesthetized, and examined thoroughly for the air holes and swellings that are indicative of the presence of large Cuterebra larvae. Dead specimens were taken by trapping and shooting during a period from August to October. They were first examined grossly, then inner surface of the skin and the underlying fascia were examined for the small first and second instar larvae which are not evident from the exterior. Larvae, following removal from the host, were maintained for experimental purposes in Syracuse dishes containing cotton wool moistened with Ringer's solution.

Larvae were fixed in K.A.A.D. solution (kerosene–alcohol–acetic acid–dioxane) and then stored in 75% alcohol–10% glycerine. Cyst tissue was taken from both male and female hosts, preserved in 10% formalin, and later sectioned and stained for histological study. First and second instar larvae were cleared in beechwood creosote and mounted in balsam. Third instar larvae were cut open along the ventral mid-line and as much of the internal organs as possible was removed. The larvae were then heated in two molar sodium hydroxide solution for two to three hours, boiled in water for 15 min., dehydrated in alcohol, and cleared in beechwood creosote. The mouth parts, stigmal plates, and a piece of the cuticle were mounted in balsam. *Cephenemyia* larvae were treated in the same fashion.

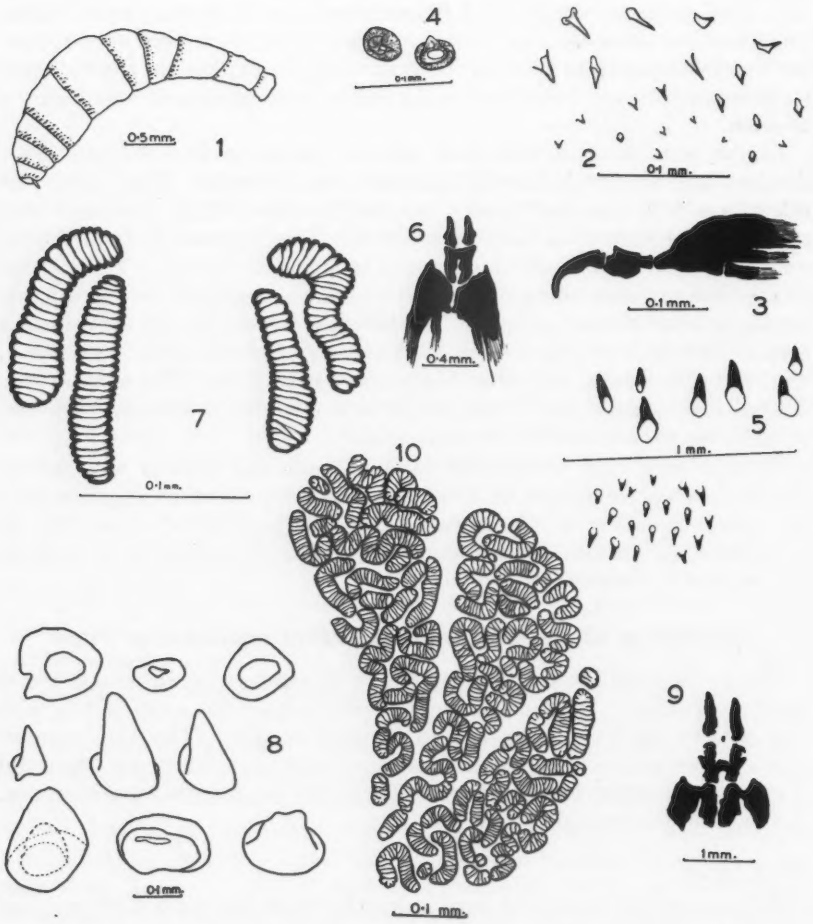
Puparia were kept individually in small vials and covered with animal charcoal to reduce fungus growth. All vials were kept at approximately saturated humidity at an average room temperature of about 24° C. Occasional applications of the commercial fungicide "moldex" were made to the exterior of the puparia.

Description of the Stages of *Cuterebra emasculator* Fitch

The larvae of all instars are composed of 12 segments of which only 10 are clearly apparent. The cephalic annulus, which bears the cephalic lobe with two sensory papillae, is superior to the oral opening. The 12th segment bears the openings of the larval respiratory apparatus, which are important diagnostic characters for each instar. The larvae are subcylindrical in section, flattened on the ventral surface.

First Instar

Larvae measure up to 2–3 mm. in length, weigh less than 0.01 gm., and are grayish-white in color. Segments two to nine bear two bands of minute spines and one or two rows of slightly larger spines (Fig. 2) on the anterior margins of each segment. Few spines occur on the last three segments (Fig. 1). The larva is generally elongate and narrow, and lacks the characteristic thickened appearance of later instars. The oral opening contains the oral hooks and the two pharyngeal sclerites, which extend back to the second (prothoracic) segment (Fig. 3, Table I). The twelfth segment bears the stigmal plates (Fig. 4). The spiracular plates and openings are so small that that they can be examined only in mounted preparations.



FIGS. 1-10. *Cuterebra emasculator.*

FIG. 1. First instar, lateral view. FIG. 2. First instar, cuticular spines. FIG. 3. First instar, cephaloskeleton. FIG. 4. First instar, posterior stigmal plates. FIG. 5. Second instar, cuticular spines. FIG. 6. Second instar, cephaloskeleton. FIG. 7. Second instar, posterior stigmal plates. FIG. 8. Third instar, cuticular spines. FIG. 9. Third instar, cephaloskeleton. FIG. 10. Third instar, posterior stigmal plate.

TABLE I

MAXIMUM STRAIGHT LINE MEASUREMENTS (IN μ) OF MOUTH PARTS OF LARVAE OF DIFFERENT INSTARS

Instar	No. measured	Oral hooks	Pharyngeal sclerites	
			First	Second
First	1	57	85	160
Second	2	138(130–147)	125(115–135)	290*
Third	6	800(757–832)	350(297–381)	490*

** The measurements of the second pharyngeal sclerite are approximate as frequently a part was destroyed when removing them from the larvae.*

Second Instar

Larvae measure up to 11 mm. in length, weigh from 0.01 to 0.1 gm. and are grayish-white in color. The first 10 segments bear two to three rows of large spines and one or two rows of minute spines on the anterior margin of each segment (Fig. 5). Segments eight, nine, and 10 also bear one or two rows of minute spines on the posterior margin of each segment. Segments 11 and 12 have spines only on the posterior margin of the segments (Fig. 11). The larva is thicker than in the preceding instar and lacks the elongate appearance. The oral hooks and pharyngeal sclerites are similar to those of the first instar, but differ primarily in the size of the latter (Fig. 6, Table I). The 12th segment is cylindrical in appearance, telescoping slightly into the 11th segment. The stigmal plates of this instar (Fig. 7) consist of slightly convoluted, paired slits, the surfaces of which are divided by fine bars. The integument is thin and transparent and much of the internal anatomy of the larva is clearly visible through it.

Third Instar

The larvae measure up to 25 mm. in length, weigh from 0.10 to 1.20 gm. They are grayish-white in color initially but change later to a reddish-brown and finally to a chocolate-brown color in the mature form. The larvae are greatly thickened in comparison to those of the preceding instars. Three longitudinal raised ridges on the lateral margins give the larva a fluted appearance (Fig. 12). Ten of the 12 segments bear approximately 21 rows of spines. The cephalic annulus and the 12th segment bear fewer spines. The spines are flat and disk-like, some with a blunt point (Fig. 8). The surface of the larva resembles coarse sandpaper. Oral hooks and pharyngeal sclerites are larger than in the preceding instars and differ mainly in the elaboration of the pharyngeal sclerites (Fig. 9, Table I). The stigmal plates, characteristic of the instar and probably of the species, are sunk in a depression and consist of three serpentine slits marked with fine crossbars (Fig. 10). The skin of the early grayish-white larva is translucent, but that of the chocolate-brown larva is opaque and extremely thick and tough.

Puparium

The puparia at time of pupation averaged 19.2 (18–21) mm. in length in 20 specimens and 0.89 (1.09–0.75) gm. in weight. The puparial case, composed of 10 visible segments covered with spines, is black in color and extremely hard. The shape of the puparium, with its flattened puparial cap, is characteristic (Fig. 14). Segment 12, bearing the larval stigmal plates, is telescoped into segment 11 and the stigmal plates are non-functional. The posterior margins of segment 11 fold over segment 12, and occasionally a small opening may exist where the folds have not met completely. The openings of the functional respiratory system of the puparium are the anterior (prothoracic) spiracles, which are mounted at the extreme anterior end of the puparium. These spiracles are present but not evaginated and do not show up on external examination in the second and third instar larvae. The puparial spiracles, resembling two spots of yellow mold, are composed of many filaments set on end in a rosette pattern, and raised about 0.5 mm. above the level of the puparial case (Fig. 14). Fig. 17 represents a longitudinal

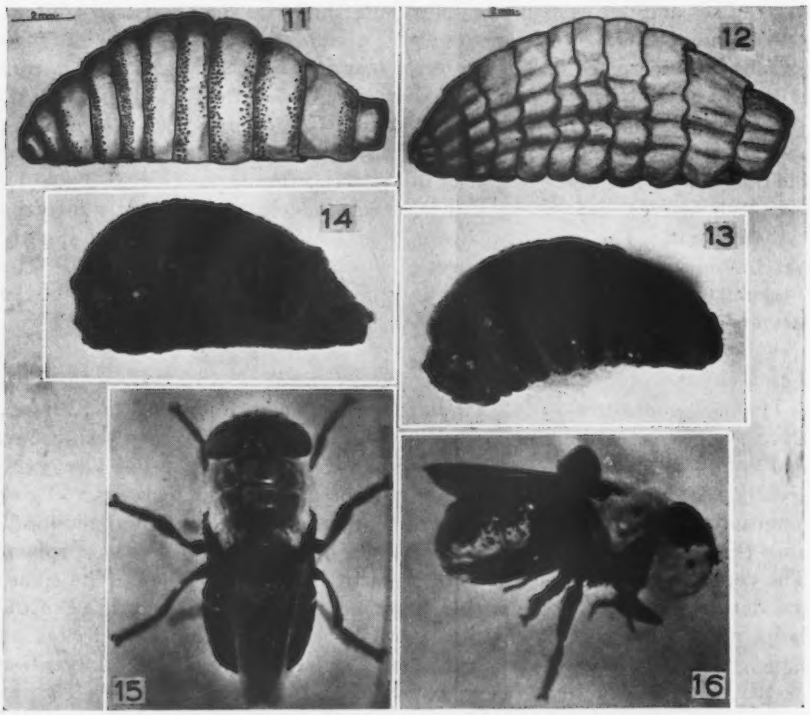


FIG. 11. *Cuterebra emasculator*, second instar, lateral view. FIG. 12. *Cuterebra emasculator*, third instar, lateral view. FIG. 13. *Oestrus ovis*, lateral view of puparium. FIG. 14. *Cuterebra emasculator*, puparium, lateral view. (Note anterior spiracles at extreme right.) FIG. 15. *Cuterebra emasculator*, dorsal view of adult. FIG. 16. *Cuterebra emasculator*, lateral view of adult.

section of the anterior spiracle which appears as a hollow cylinder, open at the top and closed at the bottom by a finely perforate plate. The inner walls and margins of the top of this cylinder are covered with fine, yellow, spiracular filaments. The filaments are hollow and appear to be composed of many small segments.

A puparial cap is present through which the adult emerges. This cap is delineated by a slightly raised ridge on the dorsal surface of segments one to four. The three longitudinal ridges, present on the lateral margins of the third instar larva, are present on the puparium.

The external view of the puparium shows that it is formed of 10 segments. The 12th larval segment, as described, is enfolded in the 11th. The prothoracic spiracles appear at the extreme anterior end of the puparia; in this region the cephalic annulus is assumed to be anterior to the spiracles but it is no longer visible on gross examination since during puparial formation it has been fused with the prothoracic segment or reduced.

Adult Fly

At the vertex, the head is broader than long, and through the middle of the antennal pit, the depth is half the width (Fig. 18). A median facial carina is present, concealed by long, fine, yellow pile. In the living specimens, the mouth parts may be visible. The genae are large, clothed in fine yellow pile, more dense on the ventral aspect. The dorsal portions of the genae, approaching the margins of the eye, are less densely clothed with pile. A pronounced, shiny-black genal tuberosity, devoid of hair or pile, is situated laterally on each side. The antennal pit is shallow, wider than long, the margins extending into the carina. The pit is dark brown, devoid of hair or pile. The antennae are three-segmented and short, the third segment larger and bearing an arista. The arista is plumose on the outer margin, the bristles continuing around the tip for a short distance. Antennae are brown. The eyes are large and brown, devoid of hair or pile, separated at the vertex by a considerable space. The margins of the eyes are also shiny brown, devoid of hair or pile. The vertex bears the ocellar plate with three ocelli, and is covered with coarse, long, black hair, forming a tuft. The posterior margin of the head is clothed with short, fine, yellow pile. The frons appears shiny-brown, but is covered sparsely with short, pollinose hairs, which are yellowish when viewed laterally, but black when viewed from the front.

The thorax (Fig. 15) is as broad as it is long and covered with dense pile. The dorsum is sparsely clothed with fine pile, appearing yellow when viewed from the side, but yellowish-black when viewed from above. Laterally (Fig. 16), in a zone enclosed by the anterior margin of the thorax, the base of the wings, and the coxa of the first two pairs of legs, the thorax is clothed in dense, long, yellow pile. On a line between the wing base and the lower margin of the eye, one-third the distance from the eye, is a tuft of long, black pile forming a conspicuous black dot. The area between the coxal joints, and laterally, beneath the wing and the scutellum, is clothed in long, black pile. The scutellum is clothed in black pile.

The abdomen is short and broad, shiny-black on the dorsum, clothed with pollinose hair in irregular patches, presenting an ash-gray appearance in certain light (Fig. 16). The fourth and fifth segments are completely clothed in fine, yellow pile. The tergites almost meet ventrally, separated by the sternites, which are covered with short, black hair. The lateroventral margins appear ash-gray with conspicuous black blotches in newly emerged specimens, but this character disappears on the ageing of both pinned specimens and the living adults.

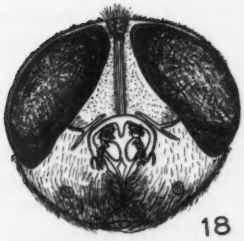
The wings are semitranslucent with a black pigmentation. The wings, when folded, project slightly over the tip of the abdomen. The alulae are large, opaque, black, in a plane at right angles to that of the wings. The following description of the venation follows the terminology used by Townsend (26). The prestigma is long, the stigma short, and the poststigma slightly longer than the stigma (Fig. 19). The radiocosta and the distocosta are about equal. Subcosta 2 and radius 1 are parallel and closely approximate to the costa for the length of the stigma. Radius 3 and 5 are also closely approximate and parallel. Radial cell 5 opens anteriorly to the wing tip. Median 3 originates at the cubitulus. Cubitus 1 does not reach the margin of the wing. The basal portions of anal 1 and 2 are strongly developed but do not continue to the margin of the wing. The squamae are large and bare.

The legs are brownish-black, clothed in fine black hair. The tarsi are five-segmented and the empodiac bristle is straight and short.

Well-developed mouth parts were found in both sexes of *C. emasculator*. A schematic lateral view of the mouth parts (Fig. 20) shows the large structure *A*, which is situated within the head, and functions apparently as a skeletal support, although the esophagus (*B*) lies in a deep channel in it. Anterior to *A* are three movable mouth parts (*C*, *D*, and *E*). The structure at *C* is hollow, flattened dorsoventrally, terminating in a sharp tip (Fig. 21). It is apparently composed of two fused elements. Inside these there is a hollow tube (*F*) which is fused to *C*. Both structures open at the distal end. Beneath the structure at *C* is the structure *D*, which is grooved, terminating distally in a sharp tip (Fig. 21). The structure at *E* represents the labial elements. These consist of a paired, lobullar glabellum distally and a support structure proximally. Within this support structure are paired glands leading to the labellum. Tracheal elements are also present. The labellar structure *E* is deeply grooved and the structures at *C* and *D* lie within it. The rods at *G* are apparently skeletal support structures. The mouth parts were observed to be everted and retracted by the fly. Fig. 22 is a diagrammatic cross section

FIGS. 17-27. *Cuterebra emasculator.*

FIG. 17. Puparium, longitudinal section of anterior spiracle. FIG. 18. Adult, frontal view of head. FIG. 19. Adult, wing. FIG. 20. Adult, lateral view of mouth parts. (*A–F*, see text.) FIG. 21. Adult, high power view of *C*, *D*, and *F*. FIG. 22. Adult, diagrammatic cross section of mouth parts. (*A–F*, see text.) FIG. 23. Adult male genitalia, lateral view. (*A–J*, see text.) FIG. 24. Adult male genitalia, anterior-dorsal view. (*A–K*, see text.) FIG. 25. Egg, lateral view. FIG. 26. Egg, high power view to show sculpturing on surface. FIG. 27. Egg, inner surface of operculum.



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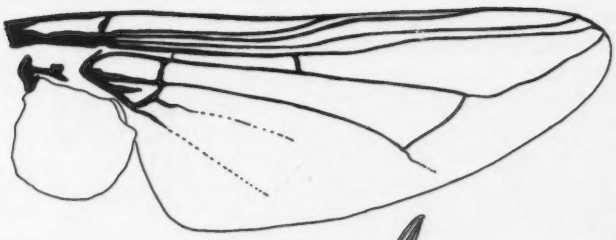

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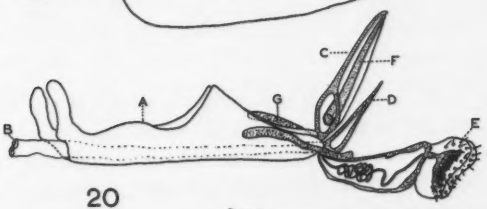

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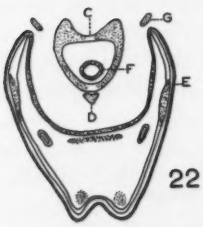

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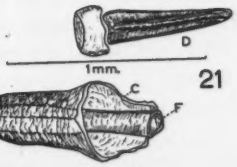

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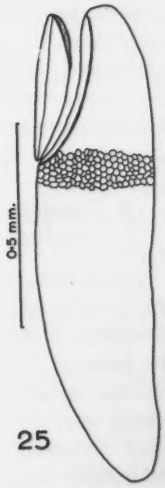

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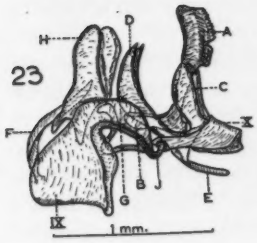

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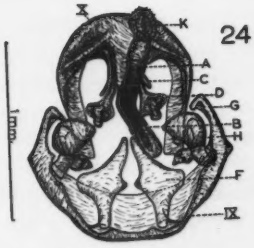

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of the mouth parts, illustrating their relationships. It will be noted that the labium is a double-walled structure and that there are no traces of the maxillary palps. The mouth parts appear to be functional and their shape suggests a piercing and lapping mechanism.

The Cuterebridae generally have been classified as belonging to the oestro-muscaris group. Townsend (26) states that the mouth parts are vestigial or lacking in the Oestridae. Since the mouth parts of *C. emasculator* differ radically from those found in the oestro-muscaris group, no homology with them has been attempted.

The male genitalia are illustrated in Figs. 23 and 24. The terminology follows that used by Snodgrass (24), but is considered to be a provisional naming of the structures. The aedeagus *A* and phallobase *B* are angled, projecting from the rest of the structures. At the tip of the serrated aedeagus is the phallotreme *K*. Surrounding the phallobase are three pairs of lobes, believed to be the lateral phallic lobes (*C*), the basal phallic lobes (*D*), and the ventral phallic lobes (*E*.) None of these stuctures appears to be movable. Segment *X* is joined firmly to segment *IX* by the lateral bars (*J*) of the latter segment. Anterior, on segment *IX*, are a pair of free lobes (*F*) which are directed posteriorly on each side of the aedeagus. Lateral to these lobes are paired movable structures (*H*) which are, perhaps, the harpogenes. The structure at *G* is the lateral process of the ninth tergite.

The Egg

The length of the egg is 1.16 (1.12–1.2) mm. and the width at the base of the operculum 0.28 (0.25–0.31) mm., on the basis of 30 specimens. The eggs are brown, subcylindrical, and flattened on the side opposite to the operculum (Fig. 25), but are blunt at the opercular end, tapering slightly toward the other. The surface is sculptured in a hexagonal pattern (Fig. 26), presenting a roughened appearance. The ovoid operculum is likewise sculptured, 0.36 (0.35–0.39) mm. in length and 0.23 (0.23–0.24) mm. at the greatest width. The lower surface of the operculum (Fig. 27) has a series of hooks situated above a mass of fibrous tissue in the egg. This is presumably the opercular release mechanism.

A Comparison of the Genus *Cephenemyia* Ltr. with Genera Included in the Families Oestridae and Cuterebridae

A large collection of larvae of *Cuterebra* and *Cephenemyia* and several adults reared from puparia has provided material for a comparative study of these two genera. Such a study was prompted by the marked differences noted in the biology of the two genera. *Cephenemyia* spp. occur only in the Artiodactyla, as do the genera generally included in the Oestridae, whereas the genera usually included in the Cuterebridae have been found in almost all other orders of mammals except the Artiodactyla, with the exception of *Dermatobia hominis* in cattle. This is a significant difference if Baer's (2) views on host specificity are accepted. A comparative study was made of the

morphology of *Cephenemyia* with that of the genera *Cuterebra* and *Dermatobia* of the Cuterebridae and the genera *Oestrus*, *Hypoderma*, *Oedemagena* of the Oestridae.

Larvae

The stigmal plates of the second and third instar *Cuterebra* larvae bear convoluted slits (Figs. 7, 10). The stigmal plates of the second and third instar *Cephenemyia* larvae are perforated (Fig. 28), as are those of the genera in the Oestridae (Figs. 29, 30). The spination of the second and third instar larvae of *Cuterebra* differs from that in the same stages of *Cephenemyia* in both structure and arrangement of spines (Figs. 5, 8). However, spine formation of *Cephenemyia* is almost indistinguishable from that found in the genus *Oestrus* (Figs. 31, 32) and is somewhat similar to that found in *Oedemagena* (Fig. 33).

Puparia

The puparia of *Cuterebra* and *Cephenemyia* show two major differences. The evagination of the anterior spiracles as the functional respiratory system of the *Cuterebra* puparia has already been described. This condition does not occur in the *Cephenemyia*, where the posterior stigmal plates of the larvae remain as the functional respiratory system of the puparia. This latter condition is found also in *Oestrus*, *Hypoderma*, *Oedemagena*, and in the Cyclorrapha in general. The flattened puparial cap of *Cuterebra* (Fig. 14) is characteristic for its group, and this flattening does not occur in the oestrid genera or *Cephenemyia* (Fig. 13).

Adults

The presence of the mouth parts in the *Cuterebra* is strikingly different from the vestigial structures found in the Cephenemyia and is dissimilar to systems in the Cyclorrapha. The wing venation of *Cuterebra* and *Cephenemyia* differs to a greater extent than does that between the *Cephenemyia* and the oestrid genera (Figs. 19, 34, 35, Table II).

A comparison was also made between the male genitalia of *Cephenemyia* and those of *Cuterebra emasculator* (Figs. 23, 24) and of genera of the Oestridae. The accessory lobes, ventral, basal, and lateral, present in the *Cuterebra*, were absent in *Cephenemyia* and *Oestrus ovis*, but the basal lobes were present, although reduced, in *Oedemagena tarandi*. The structure of the aedeagus in *Cuterebra* differed markedly from that in *Cephenemyia*, *Oestrus*, and *Oedemagena*. The structure of the aedeagus in the three latter genera was similar, and, to some extent, resembled the type of aedeagus found among the Calliphoridae.

The differences between *Cuterebra* and *Cephenemyia* are sufficient to exclude the *Cephenemyia* from the Cuterebridae, as has been done by Curran (9). The similarities between *Cephenemyia* and genera of the Oestridae justify the inclusion of the *Cephenemyia* in this family; this placement is in keeping with the views of LeClercq (18), who thought that *Hypoderma* and *Cephenemyia*

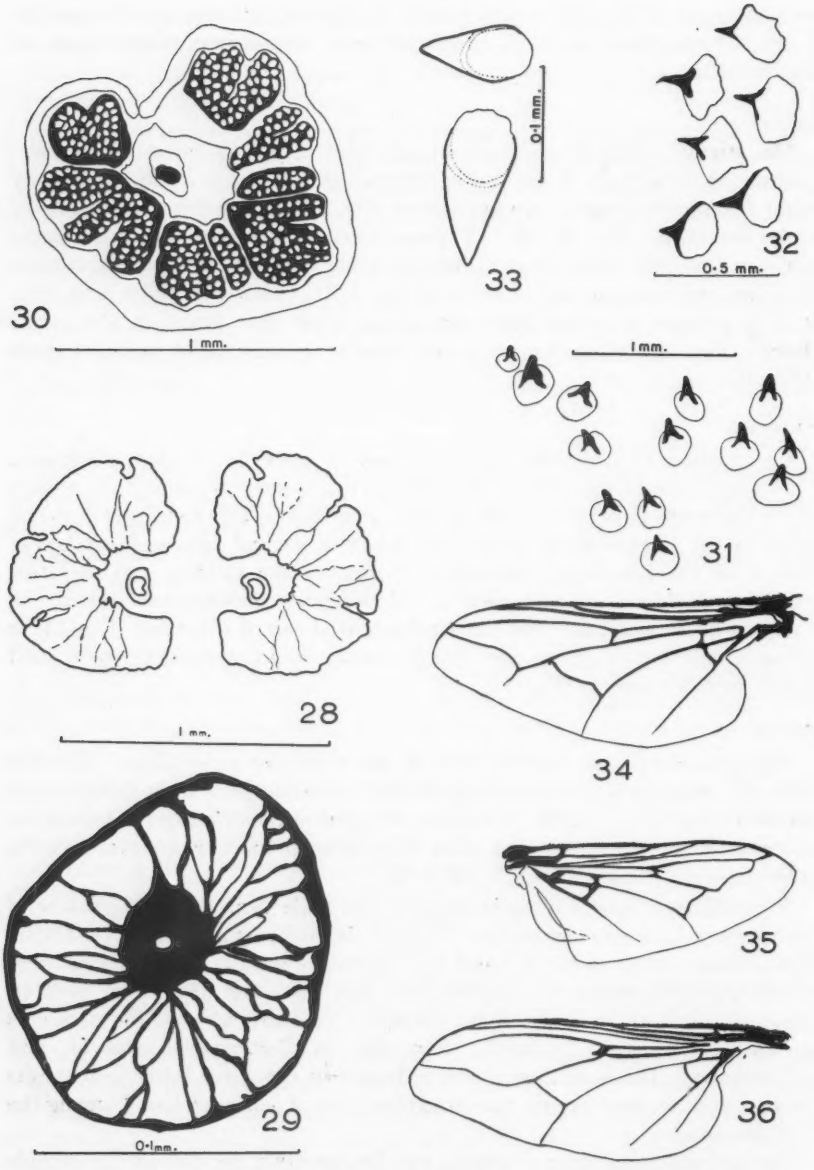


FIG. 28. *Cephenemyia phobifer*, third instar, posterior stigmal plates. FIG. 29. *Oestrus ovis*, third instar, posterior stigmal plate. FIG. 30. *Oedemagena tarandi*, third instar, posterior stigmal plate. FIG. 31. *Cephenemyia phobifer*, third instar, cuticular spines. FIG. 32. *Oestrus ovis*, third instar, cuticular spines. FIG. 33. *Oedemagena tarandi*, third instar, cuticular spines. FIG. 34. *Cephenemyia nasalis*, adult, wing. FIG. 35. *Oestrus ovis*, adult, wing. FIG. 36. *Oedemagena tarandi*, adult, wing.

TABLE II

COMPARISON OF THE WING VENATION OF THE CUTEREBRIDAE AND OESTRIDAE

Character	Cuterebridae		Oestridae	
	Cuterebra	Cephenemyia	Oestrus	Oedemagena
Prestigma	Long	Short	Long	Long
Stigma	Three-quarters of prestigma	Equal to prestigma	One-half of prestigma	Three-quarters of prestigma
Poststigma	One-half prestigma	Equal to prestigma	One-half prestigma	One-half prestigma
Radiocosta | Distocosta /	Short and equal	Distocosta is one-quarter of radiocosta	Equal	Distocosta is one-fifth of radiocosta
Subcosta 2 and radius 1	Parallel and approximate Radius 1 parallel and approximate to costa for length of stigma	Parallel but not approximate Radius 1 not parallel to costa	Not parallel or approximate Radius 1 not parallel to costa	Parallel and approximate Radius 1 not parallel to costa
Radius 3 and 5	Parallel and approximate	Not parallel or approximate	Not parallel or approximate	Not parallel or approximate
Median 3 and relation to cubitulus	At a junction or within one-eighth of cubitulus, cubitulus to radius 6	Median 3 one-third from cubitulus, cubitulus to radius 6	As in Cuterebra	As in Cuterebra
Anal veins	2–3 present	1 present	1 present	1 present
Alulae	Very large	Small	Small	Small

Terminology after Townsend (1934) in Manual of Myiology.

were related. This classification accepts the validity of the superfamily Oestroidea and Curran's concept of the family Oestridae with the addition of the genus *Cephenemyia*. The many differences observed between genera in the Cuterebridae and the Oestridae, particularly with respect to the adult mouth parts and genitalia, casts some doubt on the currently accepted inclusion of the Cuterebridae with the oestro-muscaris group.

Life History of *Cuterebra emasculator*

Larvae

No method has been available for rearing immature larvae to the puparial stage once the former have been removed from their host. In the course of the present investigation, a successful method of rearing all instars was established to provide data on their rate of development as well as to provide adult specimens.

Living *Cuterebra* larvae were obtained from chipmunks taken in the field and were transferred to captive chipmunks as follows. A captive chipmunk was anesthetized and a small incision made in the groin–belly region. A small subcutaneous pocket was formed by using a probe, and into this pocket the larva was introduced, anterior end first. The size of the opening was reduced by use of a ligature, which prevented action on the part of the host removing the larva before it was established. As many as four larvae were introduced at one time, in different subcutaneous positions in the groin–belly region of a single host. The larvae were transferred using sterile instruments, although complete asepsis was not essential for the transfer. This technique was used successfully with larvae in all stages of development, and only a few failures were experienced.

The larvae were grouped into two classes. The first, from natural infections, included those larvae which, when taken from the host, were considered almost mature and left to pupate. The second consisted of larvae that were reared to maturity by artificial transfer from their initial host to a second host. Mature larvae obtained from natural infections averaged 1.15 (1.00–1.30) gm. compared with 0.98 (0.78–1.15) gm. for those obtained following artificial transfer. The weights of the puparia formed by the two groups were the same, each group averaging 0.90 gm. Furthermore, it was assumed that little fundamental difference existed between the two groups of puparia since approximately an equal number of adults emerged from both groups.

Development of the larva was considered to be complete when it dropped from the host and a puparium of normal appearance resulted. These larvae were termed mature larvae. The weight of each larva was recorded at the time of transfer to a new host as well as at the time when it left the host, and the time interval from transfer to maturation was recorded. Therefore, the least weight increase and the shortest interval to complete development was shown by those larvae which weighed the most at time of transfer. The weight increase was plotted against time on semilogarithmic graph paper (Fig. 37), and the resulting lines of best fit indicate two rates of growth. The

point of intersection occurs at a weight comparable to the initial cuticular color change of the third instar larva. These data suggest that the second and early third instar larvae have a similar rate of growth, differing from that shown by the third instar after the cuticle color change. It may be that a third rate of growth would be found if more data were available for the first instar development. Extrapolation of the line of best fit to 1.15 gm. (the average mature weight of larvae from natural infections) suggests that larval development is completed in 18–19 days in nature.

The developmental period of two first instar larvae which were transferred and successfully reared to maturity can be compared with the developmental period of a larva assumed to have been in the first instar when the host was captured. The first larva that was transferred required 19 ± 1 days and the second required 19 days for development. The third larva, by natural infection, was so small when the living host was examined that it escaped detection, although it was noted as a small larva six days later. It was assumed that this larva was in the first instar when the host was captured. The larva matured 17 days after capture of the host. Probably one or two days should be added to the developmental period to account for the time passed in the host prior to host capture. The time required for the development of these three larvae is approximately the same, which suggests that the transfer operation has little effect on the period required for development. The rate of growth illustrated in Fig. 37 is regarded, therefore, as a true representation of the rate of development of this species.

Prepupal Stage

The mature larvae escaped from the host by enlarging the air hole in the skin. This was accomplished by a series of peristaltic movements by means of which the posterior segments of the larva were thrust to the exterior and maintained there with the aid of the cuticular spines. The margins of the air hole were torn and blood flowered freely from the wound as the peristaltic movements continued. The larva slowly forced its way out of the chipmunk, the entire process requiring 6 to 12 hours in captive animals. Since chipmunks are omnivorous, it was essential that the larva fall through the cage, for otherwise it would be eaten. Therefore the hosts were kept in cages on floors of half-inch wire mesh, which allowed the larvae to fall to a tray below.

Mature larvae were placed in sawdust, sand, charcoal, or leaf litter, in which they burrowed to various depths before they pupated. Some larvae remained on the surface with just the anterior end concealed, while others burrowed from 2 to 6 in. before pupating.

The larvae, prior to pupation, became quiescent. The first observed change was the evagination of the anterior spiracles, similar to that observed by Knipling and Brody (17) in *C. cuniculi* and *C. buccata* and Keilin (16) in *Dermatobia*. The larvae were still capable of considerable movement at this time. The mouth then became sealed off and the oral hooks, visible externally in the larval stages, could not be seen or extruded by gentle pressure. After

a varying period (usually 12–24 hr.) the larval body became contracted and the segmentation indistinct. The 12th segment, bearing the larval spiracles, was telescoped into the preceding segment, which folded over the opening and sealed it off. The anterior spiracles were now the openings of the functional respiratory system. The cuticle began to harden about this time and continued to harden for three or four days.

The shape of the puparia formed by the excised larvae and that just described for those leaving the host naturally were quite different. The former was attenuated, the segmentation distinct, with no infolding of the posterior abdominal spiracles. Although a hardened, puparium-like cuticle was formed, the anterior spiracles rarely evaginated. No morphological development was ever noted when this "puparium" was broken open and examined. It is apparent therefore that larvae must remain in the host until they are ready to pupate if a viable puparia is to be produced. This requirement of the larvae of *Cuterebra* may explain the limited success that many authors have had in rearing adults.

Puparial Period

The puparial period for 13 (of 36) puparia, which successfully completeP development at a room temperature of approximately 24° C., had a mean and median of 219 days, with a range of 134–315 days. It is in this stage that the fly overwinters.

Adult

Adults emerging from the puparia required four to five hours at 24° C. to attain normal coloration. One adult was maintained for seven days at a room temperature of 20° C., but the majority died in three to four days. During this period, the flies were observed to drink water, but not to feed on the sugar that was provided. They were generally secretive and quiescent, their activity not markedly increasing when the temperature and light intensity were raised.

Fully developed, but unfertilized, ova were obtained from five females reared in the laboratory. Two of these females were killed the day they emerged and the other females died while still in the puparia. It is apparent that the eggs are fully developed early in the adult life. This fact, taken with the short adult life span, suggests that the female is fertilized shortly after emergence, and that egg laying commences within one or two days.

Incidence of Parasitism

The incidence of *C. emasculator* in chipmunks over the period 1948–1953 is presented in Table III. The results for the individual years are not strictly comparable, as the surveys were not as intense during the period 1948–1951 as in 1952–1953, and non-infected chipmunks were frequently not recorded. These data indicate that the incidence of parasitism in the chipmunks has been at a continually high level. They further demonstrate that this parasite occurs only during the late summer.

TABLE III

INCIDENCE OF *C. emasculator* IN CHIPMUNKS, ALGONQUIN PARK, 1948–53

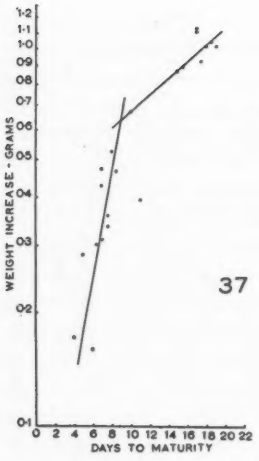
Year	No. of chipmunks	Infected chipmunks	Chipmunks examined from date of first infection, August 1st ± 3 days	
			Total	Infected
1948	2	2	2	2
1949	15	11	15	11
1950	24	8	12	8
1951	3	2	3	2
1952	46	24	37	24
1953	375	94	255	94
Total	465	141	324	141

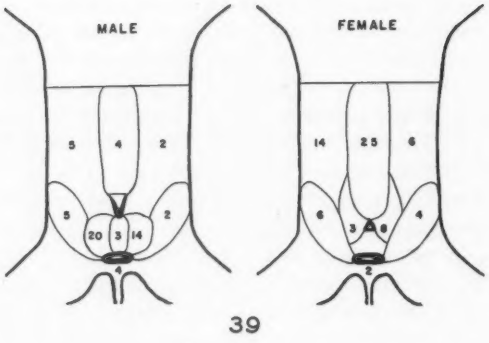
The first chipmunk infected with *Cuterebra* was taken on August 1st, in 1952, and on July 30th, in 1953. The larva taken on August 1st, 1952, pupated the same day. The first larva taken in 1953 was in the third instar and weighed 0.30 gm. Three mature larvae were taken between August 3rd–9th and pupated during this same period. These host animals must have been infected about July 7th–12th, if the development in the host requires 19 days. Most of the larvae taken during the first week of August were, however, in the second and early third instars and apparently had passed through 8 to 10 days of their development (Fig. 37). Infected animals were still taken as late as October 4th, 1953 (Fig. 38).

Incidence of Parasitism in Male and Female Chipmunks of Different Ages

Fitch (12) states that *C. emasculator* emasculated the male host, and he further implied that female chipmunks were not parasitized. This idea was accepted by Riley and Howard (20) and Townsend (26). The fact that females are commonly infected became apparent early in the present study. If there were a significant difference in the rate of infection between male and female hosts, this could be important in the biology of the fly. To determine if there was any such significant difference in the rates of infection, the hosts were divided into six groups on the basis of sex and age (Table IV), and these data were analyzed by a standard two-by-two chi-square analysis.

No statistical difference in the rate of infection between male and female chipmunks was found in 1952, and this was possibly due to the small sample size. The incidence of infection in 1953 is similar in males and females, if chipmunks of all ages are considered, but significant differences appear if the chipmunks are considered by age and sex groups. The adult males have a higher rate of infection than either the adult females, the juvenile females, or the subadult males. The difference between the adult males and either the subadult females or juvenile males is not significant. There is no significant





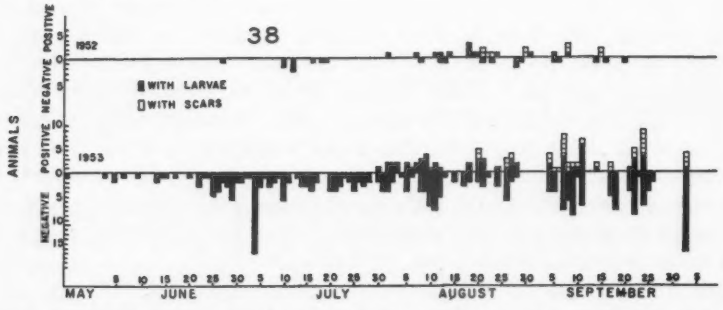


FIG. 37. Graph to demonstrate two rates of growth of the larvae of *C. emasculator*.
FIG. 38. Incidence of *C. emasculator* in the chipmunk population for years 1952 and 1953.
FIG. 39. Diagrams of the ventral surface of male and female chipmunks to illustrate the position of larvae of *C. emasculator*.

TABLE IV

COMPARISON OF INCIDENCE IN CHIPMUNKS OF DIFFERENT SEXES AND AGES

	Totals		Non-infected		Infected	
Host	1952	1953	1952	1953	1952	1953
Adult male	13	31	3	12	10	19
Subadult male	11	61	4	42	7	19
Juvenile male		27		16		11
Adult female	7	45	3	33	4	12
Subadult female		56	3	32	3	24
Juvenile female		35		26		9
Total	37	255	13	161	24	94
Total males	24	119	7	70	17	49
Total females	13	136	6	91	7	45

difference in rate of infection between any of the other combinations of groups except between the subadult females and the juvenile females, but the reason for this is not readily apparent.

The fact that the rate of infection between the total males and females is not significantly different, but the rate of infection in the adult males is higher than in any other group, can be explained on the basis of host activity and not, as Fitch (12) implies, by the fly deliberately seeking out and ovipositing on male hosts. The adult males show the greatest activity of all groups as they are the only animals competing for mates during the late summer breeding season. The adult females are caring for the juveniles which are born during the first weeks of August. The adult females and juveniles do not reach full activity until late August or early September. Thus they are not exposed to the same degree of infection as are the other groups. The subadult groups are more active than the previous groups but do not have the added activity of the breeding season. Therefore, the increased activity and exposure to infection of the adult male could lead to a higher incidence in this group.

Host–Parasite Relations

Certain species of *Cuterebra*, such as *C. americana* (Knipling and Brody, 17), *C. latifrons* and *C. approximata* (Vorhies and Taylor, 27), *C. beameri* (Beamer *et al.*, 5), and *C. lepusculi* (Ryckman, 22), are frequently found as multiple infections of four to eight larvae per host. In the present study of 113 infected chipmunks, 76 had one larva, 23 had two larvae, eight had three larvae, and the remaining six chipmunks had four to eight larvae. It is apparent that multiple infections with *C. emasculator* occur less frequently than those produced by some other species.

Observations in 1952 suggested that the parasites occupied certain parts of the host more often than others, and in this respect differed from other species of *Cuterebra* such as *C. latifrons* and *C. lepusculi* (Vorhies and Taylor, 27) and *C. beameri* (Beamer *et al.*, 5). Additional observations were made in 1953 to determine more precisely the location of larvae of *C. emasculator* in the hosts. The positions of larvae in 120 hosts are summarized in Fig. 39.

Forty-eight of the 59 larvae in the males occupied sites in a region posterior to the penis and anterior to the base of the tail. In the females, only 23 of 68 larvae were found posterior to the urogenital opening and anterior to the base of the tail, the remainder being located in the belly region, in an area about 1 in. anterior to the urogenital opening. The larvae were found on the flanks and back in a few cases. One female chipmunk was taken in which a larva was located at the base of the tail on the dorsal side. One male was taken with infection in a similar site. Three females were taken with larvae in the flanks. No larvae were taken in those parts of the animal anterior of the xiphoid process.

Thirty-four of 59 larvae from male chipmunks were located in scrotal tissue and occurred under the skin but did not penetrate through the muscle into the scrotal sac. As the larva increased in size, the cyst bulged into the scrotal

sac, and it is easy to understand why some observers mistakenly thought the larvae were in the scrotal sac. The peak infection of *C. emasculator* was found at a time when the males were not in a breeding condition and the testes were not in the scrotum. Hence, when the larvae were excised from under the skin on the scrotum and the testes were not found, it might be concluded, erroneously, that the larvae had emasculated their hosts.

Some effects of the parasite on the host tissue were studied by means of sections of the tissue that enclosed the larvae. Cysts were taken from both male and female hosts and showed the same structure, namely, a cyst wall composed of three layers, each of variable thickness.

The first layer, next the lumen of the cavity, was composed of dense collagenous material, containing scattered fibroblasts and many heterophile leucocytes; the layer around the larger larvae was somewhat thicker than that around the smaller. The second layer was composed of less densely packed collagenous fibers and numerous heterophile leucocytes. The outer layer included a layer of compressed fibers and leucocytes that intergraded to normal connective tissue.

The connective tissue layers around the sites of infection were three to four times thicker than the connective tissue in the adjacent, uninfected areas. Two of the larval sites were situated so that one was above the other, the connective tissue being thick enough to accommodate two tiers of larvae. Each larva was encapsulated as described, with normal tissue between them. Two other larval cysts were adjacent, and these two shared the same cyst wall between them. No disturbance of the dermis was noted, but the cyst wall continued around the margins of the air hole. The inner surfaces of some cysts were coated with debris.

The inner cyst wall is apparently loosely attached to the other tissues. In the majority of the sections, this layer had been separated from the other tissue in many places, although the rest of the section did not exhibit similar characteristics. This separation was considered characteristic of the layer rather than the result of the sectioning technique. The condition just described for *C. emasculator* is in contrast to the condition described by Dalmat (10), who states that the larval cyst of *C. peromysci* was not walled, as in ox warbles, but was just an area in which necrosis of muscle tissue had occurred.

In no case was damage to the urogenital tract noted when the larva was located in close proximity to it. Furthermore, no case of emasculation was ever recorded.

Since *C. emasculator* produced definite lesions in the host, it was believed that a further measure of the effect of the larvae on the hosts might be obtained by studying the effect of the larvae on the activity and the weight of the chipmunk.

Twenty chipmunks were maintained in captivity during the summer of 1953. These animals, particularily three males in adjacent cages, developed the habit of turning somersaults, which was a useful qualitative measure of

activity. Each of the three male animals was artificially infected with three or four larvae at various times throughout August and September. The animals were observed closely during different periods of the day. Their activity was markedly curtailed for the first two days after infection, probably because of postoperational shock. After this period, their activity increased to its former level and did not decrease during the developmental period of the three or four larvae. As natural infections with three or four larvae in the same host were not common, it was concluded that in nature larvae did not seriously hamper the activity of the host. This is in contrast with the view of Dalmat (10), who suggests that the presence of larvae of *C. peromysci* makes the host an easy prey, and Molliet (19) who suggests that *C. tenebrosa* is a factor controlling rodent populations.

It was noted, however, that when the larvae matured and dropped from the host, the resulting wound, with two exceptions, became purulent and the host's activity was markedly reduced. The secondary bacterial infection had a greater effect on the host than the primary attack by the *Cuterebra*. During this survey, chipmunks bearing a total of 23 fresh larval wounds (estimated at two to three days old) were taken, 19 of which wounds were purulent. These data indicate that secondary infection of the larval sites is the usual condition in nature and is not restricted to captive animals. This secondary infection of larval sites occurs commonly amongst other animals infected with species of *Cuterebra*. Roberts (21) reported serious attacks by *Cochliomyia* and *Sarcophaga* following *Cuterebra* infections of rabbits. Vorhies and Taylor (27) reported that secondary bacterial infection of larval sites in wood rats lasted from two to three weeks.

Loss of weight may be a result of parasitism. To determine the effect of *C. emasculator* on chipmunks, the differences in weight between infected and non-infected animals were compared. The results showed no consistent differences between the weights of infected and non-infected chipmunks. In many cases, the infected chipmunks were heavier than the non-infected, but this was not consistent. In general, the difference in weight between infected and non-infected chipmunks in any one age class was not as great as the variation shown among the infected or non-infected animals themselves. Hence it can be concluded that the effect of *C. emasculator* on the weight of its host is negligible.

Host Specificity

The taxonomy of the family Cuterebridae is based, to some extent, on the host from which the larva is taken. This implies that the species belonging to this family are host specific. Many reports indicate, however, that certain species, such as *C. buccata* (Bequaert, 6), *C. americana* (French, 13; Hall, 14), and *C. fontinella* (Hall, 14), can occur in many hosts. This suggests that either host specificity cannot be used as a means of identification or that great care and attention must be paid to the identification of the larvae. Lack of specificity is suggested by reports of *C. emasculator* in a number of hosts such

as red and black squirrels (Fitch, 12; Riley and Howard, 20; Hall, 14), dog (French, 13), rabbit and cat (Hall, 14). These larvae were identified by their position in the scrotum of the dog, cat, etc., rather than by morphological characters. Hence, their identification is questioned.

The host specificity of *C. emasculator* was investigated by experiments in which larvae were transferred from chipmunks to other hosts. Sixty-two of 63 larvae transferred to chipmunks grew to maturity. These acted as controls and indicated satisfactory technique. The results of the transfer of second and third instar larvae to hosts other than chipmunks are given in Table V. It will be noted that only two larvae developed of seven transferred to red squirrels. The rate of growth and final size attained was similar to that found for development of larvae in chipmunks. The other five larvae died *in situ* and the sites became purulent, as was the case in all other animals to which larvae were transferred.

TABLE V

TRANSFERS OF LARVAE OF *C. emasculator* TO VARIOUS HOSTS

Host	No. larvae transferred	No. larvae developed	Comments
Tamiasciurus hudsonicus	7	2	Two larvae developed, five died *in situ*, the sites became purulent. Marked decrease in host activity
Peromyscus maniculatus	2	0	Larvae died *in situ*, sites became purulent. Decrease in host activity
Clethrionomys gapperi	2	0	Larvae died *in situ*, sites became purulent. One host died. Decrease in activity of other host
Microtus pennsylvanicus	1	0	Larva died *in situ*, site became purulent. Host died
Mus musculus	2	0	Larvae died *in situ*, sites became purulent. One host died
Lepus americanus	4	0	Two larvae left host within 12 hr. of transfer. Two larvae alive for seven days, then died *in situ*, sites became purulent. Some loss of host activity

The possibility of red squirrels serving as natural hosts was checked by the examination of 92 squirrels collected during that period when *C. emasculator* was prevalent in the chipmunk population. Twenty-five of these were taken in areas where 50% or more of the chipmunks were infected, and 67 from other localities. None were infected.

Further indications of host specificity are shown by the results of examinations of 933 small mammals, taken during July through September, during the last two years at the Wildlife Research station. These included 703 *Peromyscus maniculatus*, 106 *Clethrionomys gapperi*, 19 *Microtus pennsylvanicus*, 4 *Synaptomys cooperi*, 53 *Napaeozapus insignis*, 19 *Zapus hudsonius*,

5 *Glaucomys sabrinus*, 18 *Eutamias minimus*, and 6 *Lepus americanus*. *C. emasculator* was not found in any of these, although all animals were taken at a time when *C. emasculator* was present in chipmunks. It is concluded that *C. emasculator* is specific for *Tamias striatus*.

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References

1. AUSTEN, E. E. On the specimens of the genus *Cutiterebra* in the collection of the British Museum, with the description of a new genus and three new species. Ann. Nat. Hist. 15 : 377-396. 1895.
2. BAER, J. C. Ecology of Animal Parasites. University of Illinois Press. Urbana, Ill. 1951.
3. BAU, A. Versuch einer Teilung der Gattung *Cuterebra* in vier Untergattungen. Zentr. Bakt. Pararasitenk. II, 77 : 542-544. 1929.
4. BAU, A. Ueber des Genus *Cuterebra*. Konowia, 10 : 147-240. 1931.
5. BEAMER, R. H., PENNER, L. R., and HIBBARD, C. W. Some notes on the pack rat cuterebrid, *Cuterebra beameri* Hall, in Kansas. J. Kansas Entomol. Soc. 16(2) : 47-50. 1943.
6. BEQUAERT, J. Cutaneous myiasis due to *Cuterebra* in Massachusetts. Psyche, 52 : 175-176. 1945.
7. BRAUER, F. Monographie der Oestriden. Vienna. 1863.
8. COQUILLET, D. W. On *C. emasculator*, with description of several allied forms. Can. Entomologist, 30 : 9-11. 1898.
9. CURRAN, C. H. The families and genera of North American Diptera. Ballou Press, New York. 1934.
10. DALMAT, H. T. A contribution to the knowledge of the Rodent Warble Flies (Cuterebridae). J. Parasitol. 29 : 311-318. 1943.
11. DALMAT, H. T. Synonymic notes on some species of Cuterebra. Entomol. News, 54(8) : 182-183. 1943.
12. FITCH, A. Emasculating bot fly, *Cuterebra emasculator* (Diptera; Oestridae). Trans. N.Y. State Agr. Soc. 16 : 478-485. 1856.
13. FRENCH, C. *Cuterebra emasculator* in dog. J. comp. Med. 14(6) : 379. 1893.
14. HALL, M. C. *Cuterebra* larvae from cats, with a list of those recorded from other hosts. J. Am. Vet. Med. Assoc. [N. S.] 12 : 480-484. 1921.
15. JAMES, M. T. The flies that cause myiasis in man. U.S. Dept. Agr. Misc. Publ. No. 631. 1947.
16. KEILIN, D. Respiratory systems and respiratory adaptations in larvae and pupae of Diptera. Parasitology, 36(1, 2) : 1-66. 1944.
17. KNIPLING, E. F. and BRODY, A. L. Some taxonomic characters of cuterebrine (Diptera) larvae, with larval descriptions of two species from Georgia. J. Parasitol. 26 : 33-43. 1940.
18. LECLERCQ, M. Revision des Oestrides de Belgique (Diptera). Bull. roy. Hist. nat. Belg. 24(41) : 1-11. 1948.
19. MOLLIET, T. K. Some observations on the life history of *Cuterebra tenebrosa* Coquillet. Contrib. No. 2198; Div. Entomol. Science Service, Dept. Agr., Ottawa. 1950.

20. RILEY, C. V. and HOWARD, L. O. On the emasculating bot fly. Insect Life, 1 : 214-216. 1889.
21. ROBERTS, R. Myiasis in jack rabbits, *Lepus californicus texianus*. J. Parasitol. 38 : 102-104. 1931.
22. RYCKMAN, R. E. *Cuterebra latifrons* reared from *Neotoma fuscipes macrotis*. Pan.-Pacific Entomol. 29 : 155-157. 1953.
23. SABROSKY, C. Personal communication. 1953.
24. SNODGRASS, R. E. Principles of insect morphology. McGraw-Hill Book Company, Inc., New York. 1935.
25. TOWNSEND, C. H. T. A synoptic revision of the *Cuterebridae*, with synonymic notes, and descriptions of one new species. Insect Inscit. Menst. 5 : 23-28. 1917.
26. TOWNSEND, C. H. T. Manual of Myiology. In twelve volumes. Sao Paulo, Brazil. 1934.
27. VORHIES, C. T. and TAYLOR, W. P. Life history and ecology of the white-throated woodrat, in relation to grazing in Arizona. Univ. Ariz. Technol. Bull. No. 86. 1940.

THE pH IN THE GUT AND BLOOD OF THE LARCH SAWFLY, *PRISTIPHORA ERICHSONII* (HTG.), AND OTHER INSECTS WITH REFERENCE TO THE PATHOGENICITY OF *BACILLUS CEREUS* FR. AND FR.[1]

By A. M. Heimpel[2]

Abstract

Regional pH measurements in the gut and the blood of 11 species of Hymenoptera and two of Lepidoptera were made. The larvae were examined in their later instars, after ecdysis, after starvation, or as mature larvae. The gut pH was found to change regionally during development and under these different conditions, but the blood pH tended to remain relatively unchanged. The pH in the gut and of the blood of the larch sawfly was found to be close to the optimum for good growth of *B. cereus* and was within the optimum activity range of the enzyme lecithinase in the anterior two thirds of the mid-gut and in the blood. This apparently holds for most of the sawfly species examined and for *Carpocapsa pomonella*, but not for those Lepidoptera examined herein.

Introduction

The mode of entry of bacteria into the insect body is *per os* by ingestion with the food or through the sericos into the salivary gland; their fate in the gut is dependent on the conditions therein. One factor of major importance in determining survival and extent of multiplication of bacteria in the gut is its pH, which must also govern the activity of soluble enzymes produced by the bacteria in the gut and in the haemolymph. Normally bacteria grow well over a fairly wide range of pH, but the activity of the enzymes they produce is generally restricted to a narrower range.

Stephens (5) reports that strains of *Bacillus cereus* Fr. and Fr. pathogenic for the codling moth, *Carpocapsa pomonella* L., are capable of growth within the pH range 5.0 to 8.0 (optimum pH 7.2 to 7.6). Experiments conducted here show that several strains of *B. cereus*, including the codling moth pathogens, are capable of spore germination and growth within the pH range of 5.0 to 9.3. Growth of the organism is relatively slow above pH 8.5. Thus a knowledge of the pH in the gut and haemolymph is important in a study of the invasion and mode of action of a pathogenic bacterium in an insect.

Three reviews of the literature on the pH of the blood and in the digestive tract of insects are available. Staudenmeyer and Stellwag (4) have covered the literature meticulously up to 1940. More recent data have been presented by Grayson (2) and by Waterhouse (7).

In 1950, a strain of *B. cereus* (culture number Pr-1017) was isolated from dead larvae of the larch sawfly, *Pristiphora erichsonii* (Htg.). Feeding trials, using larch sawfly larvae as test insects, revealed Pr-1017 to be relatively pathogenic (3). Investigations of the mode of action of *B. cereus* in the larch

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sawfly included a study of the pH in the digestive tract and the blood. For comparative purposes, this study was extended to include representatives of 11 species of Hymenoptera and two of Lepidoptera.

Materials and Methods

Both field collected and laboratory reared larvae were examined. Experience had indicated that larvae which had been starved were more susceptible to bacterial infection than feeding larvae and that molting and mature larvae were more resistant. It was thought that the pH of the gut might be responsible for these differences and therefore determinations were made on larvae under these various conditions. Larvae were starved by keeping them in sterile glass rearing jars without food for varying periods of time.

All pH determinations were made with a model G Beckman meter equipped with a calomel electrode and a single-drop open glass electrode. Readings from the gut were taken within two minutes of dissecting the larvae and measurements of the blood were completed within a maximum of 15 sec., thus reducing the possibility of the sample altering in pH by absorption of carbon dioxide (1, 2).

Samples of blood were obtained by snipping off a proleg while the insect was held gently, between the thumb and forefinger, over the glass electrode and the blood was allowed to drip into the depression. The calomel electrode was immediately brought into contact and the reading was made. Larval digestive tracts were removed from the insects through longitudinal dorsal incisions. Each isolated gut was washed in distilled water while it was lying on filter paper, so that the excess water was drawn off immediately. The gut was then cut into sections and the material in each section was squeezed out with forceps into the depression in the glass electrode and the reading was taken.

Results

The readings obtained from normal feeding sawfly larvae are recorded in Table I. Included here are Grayson's pH determinations for the digestive tract of *Macremphytus varianus* (Nort.), the only prior report of a study of this type on sawflies (2). In all species examined there are distinct differences in pH in two or three mid-gut regions. Within the group of sawfly species examined here, six general types of relationship between pH regions of the mid-gut have been established (Table I and Fig. 1). Within each of these types, all species have a similar relationship between the pH of the mid-gut regions and the gross regional anatomy of the mid-gut[3]. Differences in pH

[3] *Early in this investigation it was noted that the gross mid-gut anatomy of P. erichsonii appeared to be correlated with the pH regions of the mid-gut contents. Examination of other species revealed a similar relationship. Miss D. E. Maxwell, formerly of this laboratory, recently completed a detailed study of the internal anatomy of sawfly larvae, and examination of her drawings revealed differences in mid-gut anatomy corresponding to differences in regional pH. Miss Maxwell has pointed out that histologically the mid-gut portions are different, which suggests that the function of the histologically-distinct mid-gut regions is responsible for the variation in pH of their contents.*

TABLE I

THE pH OF THE BLOOD AND IN THE GUT OF NORMAL FEEDING LARVAE

Species	Number examined	Instar	Blood pH	Crop pH	Mid-gut pH			Hind-gut and rectum pH	Type
					Anterior	Median	Posterior		
HYMENOPTERA									
Diprion (Diprion) similis (Htg.)	20	6	6.70†		7.45	7.45	8.25	6.60	
			6.65–6.73	—	6.38–6.58	7.11–7.68	8.12–8.36	6.56–6.68	I
Neodiprion abietis Harris	23	6	6.59		6.24	8.44	7.39	6.17	
			6.47–6.53	—	6.18–6.32	7.96–8.52	7.13–7.60	6.16–6.36	II
Neodiprion americanus banksianae Rohwer	19	5	6.54		6.60	7.65	7.75	6.32	
			6.50–6.57	—	6.50–6.80	7.50–7.74	7.60–8.10	6.20–6.45	III
Neodiprion lecontei Fitch	18	6	6.88		6.61	7.49	8.29	7.00	
			6.83–6.93	—	6.54–6.70	7.32–7.55	8.22–8.43	6.84–7.32	I
Neodiprion sertifer (Geoffr.)	23	6	6.81		7.18	8.14	7.56	6.04	
			6.77–6.83	—	7.10–7.26	7.83–8.16	7.49–7.63	5.87–6.18	II
Neodiprion virginiana Rohwer	18	5	6.84		6.74	7.97	7.71	6.47	
			6.80–6.87	—	6.64–6.81	7.90–8.17	7.50–7.81	6.43–6.51	II
Diprion (Gilpinia) hercyniae (Htg.)	11	5	6.50		7.66	7.04	8.35	6.66	
			6.45–6.57	—	7.50–7.88	7.00–7.13	8.16–8.50	6.43–6.85	V
Pikonema alaskensis Rohwer	10	5	6.55		7.75		7.56	6.93	
			6.42–6.58	—	7.12–7.90	—	7.43–7.75	6.57–73.0	?
Hemichroa crocea Fourcroy	17	5	6.69		6.61	6.79	6.79	5.72	
			6.66–6.73	—	6.56–6.64	6.73–6.83	6.70–6.84	5.46–5.85	VI
Pristiphora erichsonii (Htg.)‡	44	4	6.49		7.61		8.38	6.11	
			6.45–6.57	—	7.18–7.83	—	7.42–8.50	6.00–6.71	?

* *Mean.* † *Range.*
‡ *Records so marked were made in 1952. The balance of the insects were examined in 1953.*
§ *From Grayson (2).*

TABLE I—*Concluded*

THE pH OF THE BLOOD AND IN THE GUT OF NORMAL FEEDING LARVAE—*Concluded*

Species	Number examined	Instar	Blood pH	Crop pH	Mid-gut pH			Hind-gut and rectum pH	Type
					Anterior	Median	Posterior		
HYMENOPTERA—*Concluded*									
Pristiphora erichsonii (Htg.)‡	59	5	6.64 6.58–6.72	—	7.41 7.23–7.55	—	8.43 8.09–8.59	6.04 5.73–6.20	?
Pristiphora erichsonii (Htg.)	28	5	6.64 6.61–6.69	—	7.21 7.09–7.29	7.15 7.10–7.21	8.33 8.20–8.40	6.13 5.92–6.20	V
Nematus (*Pteronidea*) *ribesii* (Scop.)	20	6	6.67 6.63–6.69	—	7.83 7.75–7.88	8.72 8.63–8.93	8.72 8.50–8.89	6.67 6.70–6.83	IV
Macremphytus varianus (Nort.)§	10–18	?	— —	5.5 5.1–5.9	6.6 6.3–7.2	—	7.1 6.3–7.7	(Rectum) 6.1 5.7–6.4	(?)
LEPIDOPTERA									
Malacosoma disstria Hbn.	31	5	6.71 6.65–6.79	6.75 6.26–7.51	9.66 9.34–9.82	10.17 9.98–10.40	9.23 8.96–9.40	5.57 5.33–5.88	
Malacosoma disstria Hbn.	20	6	6.74 6.59–6.76	6.74 6.39–7.82	9.62 9.19–9.99	10.31 10.19–10.41	9.55 9.10–10.00	5.16 5.29–7.62	
Bombyx mori Linn.	8	5–6	6.77 6.70–6.83	6.86 6.83–6.90	9.36 9.27–9.42	10.31 10.16–10.42	9.85 9.80–9.93	6.58 6.52–6.66	

* *Mean.* † *Range.*
‡ *Records so marked were made in 1952. The balance of the insects were examined in 1953.*
§ *From Grayson (2).*

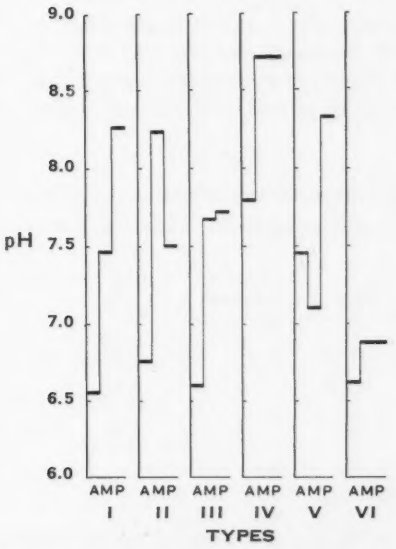

FIG. 1. The distribution of pH regions in the mid-guts of several species of sawflies; (*A*) anterior mid-gut, (*M*) median mid-gut, (*P*) posterior mid-gut. The types I to VI are referred to in Table I and most are representative of two or more species.

and regional anatomy of the mid-gut exist between types. The pH of the blood of all normal feeding larvae examined, in contrast to the pH of the mid-gut contents, is remarkably similar (range 6.49 to 6.88).

The mid-gut in molting larch sawfly larvae is usually devoid of food and rather fragile. Two pH regions are found in the mid-gut and the contents usually change to a relatively uniform pH of from 8.0 to 8.5 (see Table II). The fluid in the hind-gut becomes alkaline and the blood closer to neutral.

In mature larvae examined just before cocoon spinning (see Table III), the gut is a thin, fragile tube filled with a viscous, brown fluid. It was established that the pH values in the mature larval mid-gut were similar throughout and consequently one reading sufficed for the whole mid-gut of each specimen. In *P. erichsonii* and *Nematus ribesii*, the mid-gut pH changes towards the acid side and becomes more uniform. A drop in mid-gut pH occurs in *Neodiprion*

TABLE II

THE pH OF THE BLOOD AND IN THE GUT OF LARCH SAWFLY LARVAE DURING OR SHORTLY AFTER MOLTING

Instar	Number examined	Blood pH	Mid-gut pH		Hind-gut and rectum pH
			Anterior	Posterior	
4–5	14	6.88* 6.87–6.92†	8.23 8.00–8.33	8.41 8.12–8.51	7.68 7.55–7.80

* *Mean.* † *Range.*

banksianae, although the mid-gut still remains alkaline. The blood becomes slightly more acid in *P. erichsonii* and less acid in *N. banksianae*. The hind-gut usually becomes less acid in *P. erichsonii* and slightly alkaline in *N. banksianae*. The hind-gut of *N. ribesii* remains close to the normal pH.

TABLE III

THE pH OF THE BLOOD AND IN THE GUT OF MATURE LARVAE

Insect	Instar	Number examined	Blood pH	Mid-gut pH	Hind-gut and rectum pH
Pristiphora erichsonii	5	31	6.49* 6.46–6.54†	6.52 6.46–6.63	6.46 6.38–6.50
Neodiprion banksianae	5	19	6.65 6.63–6.69	7.46 7.00–7.77	7.23 7.10–7.32
Nematus ribesii	6	10	6.45 6.42–6.49	6.64 6.63–6.67	6.71 6.66–6.77

* *Mean.* † *Range.*

Records of the pH in the gut and blood of normal feeding larvae of *Malacosoma disstria* Hbn. and *Bombyx mori* L. are also presented in Table I. The mid-guts of these species of Lepidoptera are quite alkaline (range 8.96 to 10.42).

The mid-guts of lepidopterous and sawfly larvae undergo marked changes during starvation (see Table IV). The digestive tract empties of food and becomes fragile. Apparently there is a tendency for starvation to cause the mid-gut to become more uniform in pH. This is possibly due to a partial cessation of digestive processes and to an increased fluidity of gut content that allows greater mixing. Prolonged inanition (96 hr.) causes the pH in the *M. disstria* mid-gut to become less alkaline (range 8.10 to 9.41); the hind-gut and rectum pH becomes less acid and the blood pH slightly more acid.

Discussion

The results show that there is considerable variation in mid-gut pH of the normal feeding insects examined. The mid-gut pH of the Hymenoptera tested is consistently lower (range 6.18 to 8.93) than that of the Lepidoptera examined (8.96 to 10.42). Generally, the pH of the sawfly mid-guts should allow good growth of *B. cereus*. This is not the case in the Lepidoptera tested herein, since a pH range from 9.0 to 10.0 restricts multiplication of most known strains of this spore-former. Consequently the strains of *B. cereus* that are pathogenic for the larch sawfly have no deleterious effect when fed to *M. disstria*. However, if these same strains are injected into the blood of this insect (mean pH 6.71), they cause mortality within 18 hr.

Strains of *B. cereus* are known that kill the codling moth, *C. pomonella*, and significantly the codling moth has an unusually low mid-gut pH, ranging from 7.5 to 8.0, (5).

TABLE IV

THE EFFECT OF STARVATION ON THE pH OF THE BLOOD AND IN THE GUT OF LARVAE OF SEVERAL INSECTS

Insect	Number examined	Blood pH	Crop pH	Mid-gut pH			Hind-gut and rectum pH
				Anterior	Median	Posterior	
Starved for 3 days *Pristiphora erichsonii*	20	7.07* 6.99–7.14†	—	7.30 7.01–7.40	8.35 8.47–8.58	7.31 7.11–7.44	6.98 6.87–7.11
Starved for 17 hr. *Diprion (Gilpinia) hercyniae*	28	6.54 6.51–6.62	—	6.75 6.66–6.90	6.91 6.63–7.08	7.47 7.30–7.77	6.51 6.44–6.68
Starved for 24 hr. *Malacosoma disstria*	10	6.64 6.61–6.68	9.29 8.93–9.60	9.53 9.48–9.60	10.12 9.90–10.30	9.73 9.69–9.82	6.12 5.89–6.28
Starved for 96 hr. *Malacosoma disstria*	23	6.25 6.10–6.37	8.26 8.20–9.30	9.29 9.07–9.41	8.40 8.10–8.60	8.31 8.10–8.72	6.30 6.19–6.41

* *Mean.* † *Range.*

Starvation lowers the mid-gut pH of *M. disstria* and it is postulated that this reduction of alkalinity of the mid-gut allows germination and growth of *B. cereus* to proceed. The mid-guts of *M. disstria* larvae, starved for four days, regain the normal pH within 10 hr. after return to food. Therefore the success of the bacteria in the starved insect would depend on the numbers initially present in the mid-gut as well as the length of the starvation period.

Evidently the changes that occur in the mid-gut contents and the blood of molting and mature larch sawfly larvae are not great enough to explain the apparent resistance to bacterial infection while the larvae are in this condition.

Growth of *B. cereus* in the mid-gut is apparently not the only factor that is dependent upon the pH of the mid-gut contents involved in the pathogenicity of the bacterium. Recently it was postulated (6) that the lecithinase produced by *B. cereus* is connected with the pathogenicity of the bacterium for insects. Independently evidence has been obtained by the writer that lecithinase produced by *B. cereus* is in part responsible for the pathogenicity of some strains for *P. erichsonii*. These data will be presented in a forthcoming paper. *B. cereus* lecithinase has an optimum pH activity range of from 6.6 to 7.4. Other things being equal, the pH in the mid-gut of several of the sawflies species reported on herein should allow good growth of *B. cereus* and lecithinase activity. Investigations are under way to test the susceptibility of these sawfly species to *B. cereus* infection.

Thus it would seem that the pH of the mid-gut of insect larvae is an important factor influencing the susceptibility or resistance of the host to invasion by certain strains of *B. cereus*.

Acknowledgments

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References

1. DUSPIVA, F. Die Verwendung der Glaselectrode zur Bestimmung der H^+ Konzentration im Darmsaft der Klieder und Wachsmottenlarve. Compt. rend. trav. lab. Carlsberg, 21 : 165-167. 1936.
2. GRAYSON, J. M. Acidity-alkalinity in the alimentary canal of twenty insect species. Virginian J. Sci. 2 : 46-59. 1951.
3. HEIMPEL, A. M. A strain of *Bacillus cereus* Fr. and Fr. pathogenic for the larch sawfly, *Pristiphora erichsonii* (Htg.). Can. Entomologist, 86 : 73-77. 1954.
4. STAUDENMEYER, T. and STELLWAAG, F. Über die Wasserstoffionenkonzentration und das Pufferungsvermögen des Darmes von *Clysia ambiguella*, *Polychrosis botrana* und einigen anderen Insekten sowie ihres Futters. Z. angew. Entomol. 26 : 589-607. 1940.
5. STEPHENS, J. M. Disease in codling moth larvae produced by several strains of *Bacillus cereus*. Can. J. Zool. 30 : 30-40. 1952.
6. TOUMANOFF, C. Description de quelques souches entomophytes de *Bacillus cereus* Frank. et Frank., avec remarques sur leur action et celle d'autres bacilles sur le jaune d'œuf. Ann. inst. Pasteur, 85 : 90-98. 1953.
7. WATERHOUSE, D. F. The hydrogen ion concentration in the alimentary canal of larval and adult Lepidoptera. Austral. J. Sci. Research, Series B, 2 : 428-437. 1949.

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